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Control of plant diseases with secondary metabolite of *Clitocybe nuda*

Research Paper

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Culture filtrates of five strains of *Clitocybe nuda* displayed various degrees of antimicrobial activity against plant pathogenic fungi and/or bacteria tested. The culture filtrate of *C. nuda* strain LA82 was very effective in reducing the incidence of Phytophthora blight of pepper caused by *Phytophthora capsici* and the incidence of leaf spot on pepper caused by *Xanthomonas axonopodis* pv. *vesicatoria*. The inhibitory substance in the *C. nuda* strain LA82 culture filtrate was stable at low and high pH. It was also stable at high temperature. The inhibitory substance was dialyzable in the membrane tubing with molecular weight cut-off of 1000 but not 500 or 100. It was also exchangeable by anion but not cation exchange resins, indicating that the inhibitor has a molecular weight between 1000 and 500, and negative charge on its molecule. The inhibitor is a hydrophilic compound, but not a protein.

Introduction

Synthetic pesticides have been used heavily in agriculture to control pests and improve crop yields [1,2]. However, resistance to fungicides in current use by plant pathogens has stimulated a search for more effective disease control materials [3]. Basidiomycetes are known to produce a large number of biologically active compounds [4,5] with antibacterial, antifungal, antiviral, cytotoxic or hallucinogenic activity [6–10]. Several compounds isolated from wild macrofungi (mushrooms) also showed strong inhibition against the growth of a large spectrum of saprophytic and phytopathogenic fungi [4,11,12].

Edible macrofungi such as *Hericium erinaceum* [13], *Lentinus edodes* [14], *Flammulina velutipes* [15] and *Ganoderma lucidum* [16] were reported to be antagonistic to human pathogens. Most investigations with macrofungi have focused on the therapeutics of human disorders and less on the plant disease control [17–19]. A project was, therefore, initiated to test the culture filtrates of edible mushrooms for their ability to control plant diseases. During the survey, it was found that culture filtrates of *Clitocybe nuda* (wood blewit) were strongly inhibitory to certain plant pathogenic fungi and bacteria. The objectives of this study were to determine antimicrobial activities of culture filtrates of *C. nuda*, to evaluate their potential for controlling plant diseases, and to characterize the inhibitory substance in the culture filtrate.

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Materials and methods

Bacterial sources

Seven plant pathogenic bacteria (Table 1) used in this study were isolated from infected plants and purified by single colony on nutrient agar (1% peptone, 0.5% NaCl, 0.3% beef extract and 2.5% agar). Pure cultures of *Acidovorax avenae* subsp. *citrulli* (Schaad *et al.*) Willems *et al.* (causal agent of bacterial fruit blotch of watermelon) (strain AAC33), *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Hauben *et al.* emend. Gardan *et al.* (causal agent of soft rot of calla lily) (strain ECC), *Erwinia chrysanthemi* Burkholder *et al.* (causal agent of soft rot of calla lily) (strain ECH), *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (causal agent of wax apple bacterial wilt) (strain PS152), *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (causal agent of black rot of cabbage) (strain XCC79), *X. oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* (causal agent of bacterial blight of rice) (strain XF89-6) and *X. axonopodis* pv. *vesicatoria* (Doidge) Dowson (causal agent of bacterial spot of pepper) (strain XV64) were maintained on nutrient agar in

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TABLE 1

List of fungal and bacterial plant pathogens used in this study and their hosts

Plant pathogen	Strain	Host plant	Source[a]
Fungi			
Alternaria brassicicola	ABA01	Chinese cabbage	1
Colletotrichum higginscanum	CH01 (PA01)	Chinese cabbage	1
Fusarium oxysporum* f. sp. *lactucae	FOL (LFO1-13)	Lettuce	1
Phytophthora capsici	PCM81	Pepper	2
Bacteria			
Acidovorax avenae* subsp. *citrulli	AAC33	Watermelon	3
Pectobacterium carotovorum* subsp. *carotovorum	ECC (ZI1)	Calla lily	3
Erwinia chrysanthemi	ECH (Cas7)	Calla lily	3
Ralstonia solanacearum	PS152	Wax apple	3
Xanthomonas campestris* pv. *campestris	XCC79	Cabbage	3
Xanthomonas oryzae* pv. *oryzae	XF89-6	Rice	4
Xanthomonas axonopodis* pv. *vesicatoria	XV64	Pepper	3

[a] Source of plant pathogens: 1, from the Laboratory of Plant Disease Management, National Chung Hsing University (NCHU), Taiwan; 2, from the Laboratory of Phytophthora Biology, NCHU; 3, from the Laboratory of Plant Bacterial Diseases, NCHU; and 4, from the Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan.

the laboratory. Before use, all bacterial strains were cultured in nutrient broth (Difco, USA) on a rotary shaker (Firstek, Taiwan) at 30 °C. After 24 to 48 h, bacteria were harvested by centrifugation and resuspended in sterile distilled water. The concentrations were adjusted by measuring the optical density at 600 nm (OD_{600} = 0.3, equivalent to 10^8 CFU/ml) with a spectrophotometer (Biotech photometer, UK).

Strains of ***C. nuda***

The strains of *C. nuda* used in this study consisted of strains 999, LNG, LNE2, LA82 and LA84. All of them were obtained from Taiwan Agricultural Research Institute, and maintained on compost extract agar (CEA) consisting of compost extract and 2% agar. Compost extract was prepared by heating 2% rice straw compost [20] and 1.5% corn meal at 100 °C for 30 min and filtering the mixture through two layers of cheesecloth.

Spore production of test organisms

Plant pathogenic fungi used in this study were listed in Table 1. They were isolated from infected plant materials and maintained on potato dextrose agar (PDA) and 20% V-8 agar consisting of 20% V-8 juice, 0.3% $CaCO_3$ and 2% agar. Sporangia of *Phytophthora capsici* Leonian (isolate PCM81) were produced by growing the organism on 20% V-8 agar at 24 °C in a 12-h light–dark cycle, with cool white fluorescent irradiation for seven days. For release of zoospores from sporangia, the culture was cut into small pieces (ca. 10 × 10 mm), transferred to 15 ml sterile distilled water in a 9-cm Petri plate, and incubated under light. After two days at 24 °C, the water was replaced with 15 ml of sterile distilled water and the Petri plate was placed in a refrigerator at 4 °C for 2 h. Conidia of *Alternaria brassicicola* (Schwein) Wiltshire (isolate ABA01), *Colletotrichum higginscanum* Sacc. in Higgins (isolate CH01) and *Fusarium oxysporum* f. sp. *lactucae* Matuo et Motohashi (isolate FOL) were produced on PDA. These fungi were incubated at 24 °C under 12 h illumination for 14 days. The concentration of spore suspension was adjusted to 10^5 spores/ml with a Pipetman microlitre pipet [21] for all microorganisms tested, except *C. higginscanum*, which was adjusted to 10^4 spores/ml to avoid self-inhibition [22].

Preparation of culture filtrates

A culture block (10 mm × 10 mm × 3 mm) of *C. nuda* was placed in 100 ml potato dextrose broth (PDB, Difco) in a 500-ml flask and incubated at 24 °C for 21 days on a shaker. The cultural fluid was filtered through a Whatman No. 1 filter paper. The filtrate was sterilized by filtration through a 0.22 μm filter (Millipore, USA) and stored at −20 °C.

Spore germination tests

To test culture filtrates for inhibition of spore germination, 10 μl of spore suspension was mixed with an equal volume of culture filtrate in a cavity of a sterile eight-cavity slide. Slides were kept moist by placing them on L-shaped glass rods on moistened paper towels in 9-cm plastic Petri dishes sealed with Parafilm. A spore suspension mixed similarly with PDB was used as a control. Germination was recorded after incubation at 24 °C for 8 h and 100 spores were counted for each of the four replicates. All the experiments were repeated twice.

Effects of culture filtrates on mycelial growth of fungi

The effects of *C. nuda* culture filtrates on mycelial growth of *A. brassicicola*, *C. higginscanum*, *F. oxysporum* f. sp. *lactucae* and *P. capsici* were determined using 2% (w/v) PDA. A PDA plate was flooded with 200 μl of a culture filtrate to form a thin film. For controls, each plate was flooded with 200 μl of sterile distilled water. Culture disk (7 mm in diam.) from a 10-day-old culture was placed on the medium at the center of the Petri dish. The plates were incubated at 24 °C for seven days before measurement of colony diameter. Effects of culture filtrates were calculated according to the formula of Pandey *et al*. [23]: inhibition (%) = [(colony diameter in control − colony diameter in treatment)/colony diameter in control] × 100. Four replicates in each treatment were used and the experiment was repeated twice.

Effects of culture filtrates on growth of bacteria

Effects of culture filtrates on bacterial growth were tested in Petri plates containing 2.5% nutrient agar (NA). The 0.8% soft agar at 48 °C was inoculated with a bacterial broth culture (10^6–10^8 CFU/ml) of the test organism and poured over the NA plates. Four filter

paper disks (8 mm in diam.) (Advantec, Japan) immersed in each culture filtrate were placed on a bacterium-inoculated plate. Filter paper disks soaked in sterile PDB were used as controls. After inocubation at 30 °C for 48 h, the diameters of inhibition zones were recorded. Four replicates were used and the experiment was repeated twice.

Control of Phytophthora blight of pepper with culture filtrates

Five-week-old sweet pepper (*Capsicum annuum* L.) cv. Trim-Star (Known-You Seed Co., Taiwan) plants each with four to five fully expanded leaves were sprayed to runoff with culture filtrate of *C. nuda* (LA82). Control plants were similarly sprayed with PDB. The plants were sprayed three times over a three-day period. One day after the last leaf spray, each leaf was inoculated with four 2-μl drops of spore suspension of *P. capsici* along the edge of the leaf, and a 10-μl drop of 1% V8 juice agar at 55 to 60 °C was added to each inoculum drop [24]. Inoculated plants were placed in moist chambers and kept in the laboratory at 24 °C. After incubation at 24 °C for three days, the number of lesions that developed at the inoculated sites was recorded. Three leaves per plant were inoculated and four plants were used for each treatment. Statistical analysis was done using ANOVA and Tukey's test ($P = 0.05$). The experiment was repeated twice.

Control of bacterial spot of pepper with culture filtrate

Sweet pepper leaves sprayed to runoff with suspension of *X. axonopodis* pv. *vesicatoria* (XV64) at 10^8 CFU/ml one day after being sprayed once with culture filtrate of *C. nuda* (LA82). Sweet pepper plants sprayed with PDB and inoculated on the same day were used as the controls. Inoculated plants were incubated under moist conditions for two days before being transferred to the greenhouse. Number of lesions developed on each leaf was recorded after eight days. Disease severity was calculated by (average lesion number on treated leaves/average lesion number on control leaves) × 100. Five leaves per plant and four plants per treatment were used. Statistical analyses were done as above. The experiment was conducted twice.

Effect of adsorptive materials on the inhibitory activity

To test the nature of the inhibitor in the culture filtrate of *C. nuda* (LA82), 10 g each of Diaion SK1B cation exchange resins (equivalent to Amberlite IR-120), Diaion SA12A anion exchange resins (equivalent to Amberlite IRA-420) (Tai-Young Chemical Co., Kaohsiung, Taiwan), or activated charcoal (Sigma–Aldrich, USA) were washed with 100 ml of distilled water four times by shaking for a 6-h period to remove possible inhibitory substances [25]. Fifty milliliters of culture filtrate was shaken with either 5 g of each type of resins or 5 g of activated charcoal in a 250-ml flask at 150 rpm for 24 h and filtered through a Whatman No. 1 filter paper [25]. The treated culture filtrates were used for germination tests. Four replicates were used for each treatment and the experiments were repeated twice.

Effect of pH on the inhibitory activity

The original pH of *C. nuda* (LA82) culture filtrate was 4.5. To study the effect of pH value on the inhibitory activity of culture filtrate, 35 ml of the culture filtrate was adjusted to pH value of 2 to 12 with 1N HCl or 1N NaOH. Spore germination of *P. capsici* was used to test activity of culture filtrate as described above. PDB with a similarly adjusted pH value was used as the control. For testing the stability of the inhibitors under low or high pH conditions, culture filtrate of *C. nuda* (LA82) was adjusted to pH 2 with 1N HCl or 12 with 1N NaOH. After 1 or 24 h at 24 °C, the pH values of the culture filtrates were adjusted back to the original 4.5 [26], and the activity of culture filtrates was tested as described above. Four replicates were used and the experiments were repeated twice.

Heat treatment

Culture filtrate was treated at 60 °C for 30 min, at 100 °C for 15 min or autoclaved for 15 min. After heat treatment, activity of the culture filtrate was assayed as described above.

Molecular weight estimation of the inhibitory substances

Five milliliters of culture filtrate was placed in a dialysis tubing with a molecular weight cut-off of 100, 500 or 1000 (Spectrum Medical Industries Inc., Los Angeles, CA), and dialyzed at 24 °C against 4000 ml of distilled water that was changed four times over a 24-h period [25]. After dialysis the filtrates were tested for spore germination as described above.

Determination of the nature of the active compound

The culture filtrate of *C. nuda* (LA82) was freeze dried. Two grams of dry powder, obtained from approximately 200 ml of culture, was extracted with 40 ml of water, 95% ethanol, methanol, acetone or dichloromethane in a 250-ml flask on a shaker at 150 stroke/min for 24 h. The mixture was filtered through Whatman No. 1 filter paper. For bioassay, 10 ml extract was evaporated to dryness in a fume hood, and the residue was redissolved in 10 ml distilled water. To determine if the inhibitory substance is a protein, 10 ml ethanol extract of freeze dried culture filtrate was evaporated to dryness as described above. One milligram of the residue was processed through SDS-polyacrylamide gel electrophoresis (SDS-PAGE) following the protocol of Sambrook and Russell [27].

Results

*Effects of culture filtrates of **C. nuda** on plant pathogenic fungi and bacteria*

The culture filtrates of strains LNE2 and LA82 of *C. nuda* showed complete suppression of spore germination of *P. capsici*, while culture filtrates of strains 999 and LNG were moderately inhibitory, reducing the germination rate from 99% in the control to 23 to 59% (Table 2). Strain LA84 was innocuous to *P. capsici*. Culture filtrate of strain LA82 was also capable of complete inhibition of conidial germination of *C. higginscanum*, but culture filtrates of the other four strains were ineffective in the suppression of spore germination of this pathogen. Only the culture filtrate of strain LA82 was moderately inhibitory to spore germination of *F. oxysporum* f. sp. *lactucae*, reducing the germination rate from 100% in PDB control to 58%. Other culture filtrates were ineffective. Conidial germination of *A. brassicicola* was strongly inhibited by culture filtrate of strain LA82, but not by others. Mycelial growth of *F. oxysporum* f.sp. *lactucae* was slightly inhibited by all the culture filtrates tested, while none of the filtrates were inhibitory to that of *P. capsici* (Table 2). Three of the culture filtrates also showed slight inhibition against growth of *C. higginscanum* and *A. brassicicola*.

TABLE 2

Effects of culture filtrates of five strains of *Clitocybe nuda* on spore germination and mycelial growth of four fungal plant pathogens[a]

Strain[b]	Spore germination (%)[c]				Inhibition of mycelial growth (%)[c]			
	PCM81	CH01	FOL	ABA01	PCM81	CH01	FOL	ABA01
999	59 B[d]	91 A	100 A	80 C	0	0 B	10 B	23 A
LNG	23 C	86 B	100 A	90 B	0	5 A	19 A	0 B
LNE2	0 D	87 B	100 A	83 C	0	0 B	11 B	19 A
LA82	0 D	0 C	58 B	0 D	0	8 A	19 A	22 A
LA84	92 A	85 B	98 A	81 C	0	6 A	12 B	0 B
PDB (control)	99 A	87 B	100 A	98 A	0	0 B	0 C	0 B

[a] Abbreviations of plant pathogens were listed in Table 1.
[b] Edible macrofungi were cultured in potato dextrose broth under shaking at 125 rpm for 21 days at 24 °C.
[c] The experiments were replicated four times and repeated twice; a total of 100 spores was counted for each replicate; inhibition of mycelial growth (%) = (colony size in control − colony size in treatment)/colony size in control × 100.
[d] Values followed by the same letter in the same column are not significantly by Tukey's difference test ($P = 0.05$); the experiment was repeated twice.

TABLE 3

Effects of culture filtrates of five strains of *Clitocybe nuda* on growth of seven plant bacterial pathogens[a]

Strain[b]	Diameter of inhibition zone (mm)[c]						
	AAC33	ECC	ECH	PS152	XF89-6	XCC79	XV64
999	0	0	0	0	14 A[d]	23 A	9 A
LNG	0	0	0	0	0 B	20 A	10 A
LNE2	0	0	0	0	0 B	22 A	10 A
LA82	0	0	0	0	0 B	21 A	10 A
LA84	0	0	0	0	0 B	15 B	0 B
PDB (control)	0	0	0	0	0 B	0 C	0 B

[a] Abbreviations of plant pathogens were listed in Table 1.
[b] Edible macrofungi were cultured in potato dextrose broth under shaking at 125 rpm for 21 days at 24 °C.
[c] Four replicates were used for each treatment.
[d] Values followed by the same letter in the same column are not significantly by Tukey's difference test ($P = 0.05$); the experiment was repeated twice.

Culture filtrates of all five *C. nuda* strains were inhibitory to *X. campestris* pv. *campestris*, but none were inhibitory to *A. avenae* subsp. *citrulli*, *P. carotovorum* subsp. *carotovorum*, *E. chrysanthemi* or *R. solanacearum* (Table 3). *X. axonopodis* pv. *vesicatoria* was inhibited by all except culture filtrate of strain LA84, while *X. oryzae* pv. *oryzae* was inhibited by that of strain 999 only.

Culture filtrate of *C. nuda* strain LA82 showed complete inhibition of spore germination of three species of fungi tested and moderate inhibition against that of the other species. It was also strongly inhibitory to cell growth of two bacterial species tested. This culture filtrate was, therefore, selected for subsequent studies.

Control of Phytophthora blight and bacterial spot of pepper with culture filtrate

Spraying culture filtrate of *C. nuda* strain LA82 on pepper leaves was able to reduce the disease incidence of Phytophthora blight of pepper caused by *P. capsici* from 100% on the control to 11%. The culture filtrate was also effective in reducing the disease severity of bacterial leaf spot of pepper caused by *X. axonopodis* pv. *vesicatoria* (XV64). It was able to reduce the disease severity from 100% on the control to 43%.

Characterization of the inhibitory substance in the culture filtrate

After treatment with anion exchange resins, the culture filtrate of *C. nuda* strain LA82 became non-inhibitory to the germination of *P. capsici* zoospores (Table 4). The culture filtrate also was not inhibitory to zoospore germination after being treated with activated charcoal. Treatment with cation exchange resins did not affect the inhibitory effect of the culture filtrate. Repeated treatment with fresh cation exchange resins did not reduce the inhibitory effect (data not shown). The culture filtrate was strongly inhibitory to germination of zoospores of *P. capsici* at pH values ranging from 4.0 to 5.0 (Table 5). However, the culture filtrate was not inhibitory at pH 6.0 to 10.0. The culture filtrate remained inhibitory to zoospore germination of *P. capsici* when its pH was maintained at 2.0 or 12.0 for 1 or 24 h before being adjusted back

TABLE 4

Effects of different treatments of culture filtrate of *Clitocybe nuda* strain LA82 on its inhibitory activity against zoospore germination of *Phytophthora capsici*

Treatment	pH value[a]	Germination (%)
Culture filtrate without treatment	4.5	0
Cation extract resins	5.0	0
Anion extract resins	4.7	91
Activated charcoal	4.9	96
PDB (control)	4.5	90

[a] pH value of the culture filtrate after treatment.

TABLE 5

Effect of pH of culture filtrate of *Clitocybe nuda* strain LA82 on zoospore germination of *Phytophthora capsici*

pH	Germination (%)	
	Treatment	Control (PDB)
2.0	0 E[a]	0 F
3.0	0 E	17 E
4.0	0 E	65 B
4.5	0 E	84 A
5.0	5 D	87 A
6.0	86 A	80 A
7.0	88 A	86 A
8.0	89 A	82 A
9.0	86 A	85 A
10.0	82 A	83 A
11.0	70 B	51 C
12.0	37 C	39 D

[a] Values followed by the same letter in the same column are not significantly by Tukey's difference test ($P = 0.05$); the experiment was repeated twice.

TABLE 6

Zoospore germination of *Phytophthora capsici* in the culture filtrate of *Clitocybe nuda* strain LA82 adjusted to pH value of 2.0 or 12.0 for 1 or 24 h before being readjusted back to the original pH of 4.5

pH	Treatment period (h)	Germination (%)
2.0	1	0
2.0	24	0
12.0	1	0
12.0	24	0
PDB (control) 4.5		84

to 4.5 (Table 6). None of the heat treatments decreased the inhibitory effect of culture filtrate on zoospore germination of *P. capsici* or growth of *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria* (Table 7).The inhibitory substance in the culture filtrate failed to diffuse through the membrane tubing with a

TABLE 7

Effect of the heat treatments of culture filtrate of *Clitocybe nuda* strain LA82 on zoospore germination of *Phytophthora capsici* (PCM81) and growth inhibition of *Xanthomonas axonopodis* pv. *vesicatoria* (XV64) and *X. campestris* pv. *campestris* (XCC79)

Temperature (°C)[a]	Germination (%)	Diameter of inhibition zone (mm)	
	PCM81	XCC79	XV64
25	0	19	10
60	0	20	10
100	0	20	10
121 (autoclave)	0	20	10
Control (PDB)	84	0	0

[a] The culture filtrate was treated at 25 °C or 60 °C for 30 min, or 100 °C or autoclaved for 15 min; the experiment was repeated twice.

TABLE 8

Inhibition of germination of zoospore of *Phytophthora capsici* (PCM81) or growth of *Xanthomomas axonopodis* pv. *vesicatoria* (XV64) and *X. campestris* pv. *campestris* (XCC79) in culture filtrate of *Clitocybe nuda* strain LA82 following dialysis with membrane tubing of various molecular weight cut-offs

Molecular weight cut-off	Germination (%)	Inhibition zone (mm)	
	PCM81	XCC79	XV64
1000	86 A[a]	0 B	0 B
500	0 B	19 A	10 A
100	0 B	19 A	10 A
Control (PDB)	88 A	0 B	0 B
Nontreated culture filtrate	0 B	19 A	10 A

[a] Values followed by the same letter in the same column are not significantly by Tukey's difference test ($P = 0.05$). The experiment was repeated twice.

TABLE 9

Effectiveness of different solvents to extract inhibitory substance from freeze dried culture filtrate of *Clitocybe nuda* strain LA82 to suppress germination of zoospores of *Phytophthora capsici* (PCM81)

Treatment	Germination (%)
Water extract	0
Ethanol extract	0
Methanol extract	0
Acetone extract	67
Dichloromethane extract	71
Control (water)	69

molecular weight cut-off of 100 or 500 during dialysis (Table 8). After dialysis, the culture filtrate was still inhibitory to germination of *P. capsici* zoospores or growth of *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria*. Following dialysis in the membrane tubing with a molecular weight cut-off 1000, the culture filtrate was no longer inhibitory to zoospore of germination of *P. capsici* or growth of *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria*.

Nature of the active compound

Water, ethanol or methanol extract of the dried poweder of *C. nuda* culture completely inhibited germination of *P. capsici* zoospores. However, acetone or dicholoromethane did not affect the germination (Table 9). No protein band was found following SDS-PAGE of the inhibitory extract, indicating that the inhibitor is not a protein.

Discussion

Results from this study showed that culture filtrates from different strains of *C. nuda* displayed different inhibitory effect against the plant pathogenic fungi or bacteria tested. Production of different amounts of antimicrobial compounds by different strains of the same species of macrofungi has been reported previously [28,29]. Culture filtrate of *C. nuda* strain LA82 was inhibitory to zoospore germination of *P. capsici* and was also effective in reducing disease

caused by this pathogen. The culture filtrate likewise was strongly inhibitory to growth of *X. axonopodis* pv. *vesicatoria* and was also capable of reducing the incidence of leaf spot on pepper caused by this bacterium. Mycelial leachate of macrofungus *Lentinula edodes* also has been reported to suppress bacterial wilt of tomato and lima bean [30]. Sporophores and mycelia of *C. nuda* were reported to contain antimicrobial activity against human pathogens *Staphylococcus aureus* [29,31,32] and *Candida albicans* [29,31]. To our knowledge, this is the first report of plant disease control by culture filtrates of edible macrofungi.

The inhibitory substance in the culture filtrate of *C. nuda* was resistant to heat treatment and stable at high or low pH. The dializablity of the inhibitory substance in the culture filtrate with a molecular weight cut-off of 1000 but not 500 or 100 and its removal by anion but not cation exchange resins, indicate that the inhibitor has a molecular weight between 1000 and 500 and negative charge on its molecule. SDS-PAGE of the inhibitory substance indicated that it is not a protein. However, solvent solubility test showed that the inhibitor is a hydrophilic compound.

Since *C. nuda* is an edible mushroom, its culture filtrate is considered safe for use in the disease control by organic farmers or home gardeners. The inhibitory substance in the culture filtrate has the favorable features of being stable at high temperature or under acid and alkaline conditions. Therefore, it may be possible to develop it into an environmentally friendly commercial product for disease control of agriculture crops.

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